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Determination of cut-off values and sensitivities of tests for the diagnosis of subclinical cushing's syndrome in functional investigation of adrenal incidentalomas

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Abstract

Aim: The aim of our study was to determine the sensitivities of tests used in the diagnosis of subclinical Cushing's syndrome (SCS) in patients with adrenal incidentalomas using clinical findings and other biochemical parameters. Material and Method: One hundred and twenty-nine patients with adrenal incidentalomas who were followed up by our endocrinology clinic were included in the study. Patients were divided into 4 groups according to the 1-mg DST results and these groups were compared in terms of age, gender, hormonal parameters, and comorbidities associated with Cushing's syndrome. The cut-off value of salivary cortisol level for the diagnosis of SCS was calculated by ROC curve analysis. Result: If the cut-off values of plasma cortisol levels after the 1-mg DST were considered ≥1,8 µg/dL, ≥3 µg/dL, and ≥5µg/dL, the detection rates of SCS were respectively 22,4%, 1,4%, and 3,8%. When the cut-off value of plasma cortisol levels after the 1-mg DST was considered ≥1,8µg/dL, the sensitivity, specificity, and cut-off value of salivary cortisol levels were calculated as 68%, 57%, and 0.195 µg/dL by ROC curve analysis. Discussion: As the adrenal mass size and patient age increased, cortisol suppression decreased according to the 1-mg DST. As the cut-off value of plasma cortisol level used in the 1-mg DST decreased, the incidence of SCS increased significantly. This leads to overdiagnosis of SCS in asymptomatic patients with adrenal incidentalomas. New cut-off values should be determined for salivary cortisol level according to different populations.

Keywords

Cushing's Syndrome; Adrenal Incidentalomas; Salivary Cortisol

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Introduction

Adrenal incidentalomas are mass lesions greater than 1 cm that are detected in the adrenal gland on images taken for another reason [1]. The incidence of adrenal incidentalomas has shown a significant increase all over the world with the widespread use of imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). Although the incidence of adrenal incidentalomas increases with age, it is seen between 4-10% on average [2]. Ten to fifteen percent (%) of adrenal masses secrete hormones [3]; 7% of adrenal incidentalomas cause subclinical Cushing's syndrome (SCS). Patients who have the autonomic cortisol secretion without typical clinical signs and symptoms of Cushing's syndrome are diagnosed as SCS [4]. After the 1-mg DST performed for the diagnosis of SCS, a high false-positive rate has been obtained due to a low cut-off value of plasma cortisol level whereas a high false-negative rate has been obtained due to a high cut-off value of plasma cortisol level [5]. The aim of our study was to determine the sensitivities of tests used in the diagnosis of SCS in patients with adrenal incidentalomas by clinical findings and other biochemical parameters.

Material and Method

The data of 129 patients who were followed up for adrenal incidentaloma in the Endocrinology Clinic of Ondokuz Mayıs University Medical Faculty between the years 2012-2014 were retrospectively analyzed. The Ethics approval was obtained from the Institutional Review Board of the Hospital. This study was conducted in accordance with the Declaration of Helsinki. There is no international standardized cut-off value for plasma cortisol level after the 1-mg dexamethasone test (DST) in patients with SCS. At different medical centers, the cut-off values for dexamethasone-induced suppression of plasma cortisol levels are considered <1.8 μg/dL, <3 μg/dL, and <5 μg/dL. We examined our patients according to these three cut-off values of plasma cortisol levels.

The patients who had plasma cortisol levels <1.8 μg/dL after the 1-mg DST were considered to have a positive response to the 1-mg DST (non-SCS group). The patients who had plasma cortisol levels ≥1.8 μg/dL after the 1-mg DST were divided into 3 groups (SCS groups): Group A (=1.8-3 μg/dL), Group B (=3-5 μg/dL), and Group C (≥5 μg/dL).Patients with and without SCS were compared according to their hormonal, biochemical, and comorbidity characteristics. However, 6 patients who had plasma cortisol levels ≥5 μg/dL after the 1-mg DST were not included in the statistical evaluation. Therefore, the data were obtained by comparing the non-SCS group with Groups A and B.

Statistical analysis

The Kolmogorov-Smirnov test was performed to examine whether the data showed a normal distribution**.** The Student's t-test, Mann-Whitney U test, Kruskal-Wallis test, and Pearson's Chi-square test were used to compare the data of 3 groups. In multiple significance tests, calculations were performed with the Bonferroni correction. ROC curve analysis was used to determine the cut-off value of salivary cortisol level. The results were considered statistically significant if statistical tests were based on a p-value of ≤ 0.05 with a confidence interval of 95%. The SPSS 15.0 package program was used for all calculations.

Results

A total of 129 patients were included in the study. Of these patients, 65.9% were female and 34.1% were male. The mean age was 57 ± 10.5 years. Patients were first divided into 2 groups according to the 1-mg DST results: non-SCS (n=87) (plasma cortisol level <1.8μg/dL) and SCS (n=42) (plasma cortisol level≥1.8 μg/dL). 42 patients who had plasma cortisol levels ≥1.8 μg/dL after the 1-mg DST were then divided into 3 groups (SCS groups): Group A (n=23) (=1.8-3 μg/dL), Group B (n=19) (=3-5 μg/dL), and Group C (n=6) (≥5 μg/dL). Since there were 6 patients in Group C, these patients were evaluated in Group B.The demographic characteristics of the non-SCS and SCS groups are shown in Table 1.

All patients underwent the 1-mg DST. Twenty-three of 42 patients with non-suppressed plasma cortisol levels after the 1-mg DST underwent the 2-mg DST. After the 2-mg DST, 6 patients had plasma cortisol levels <5μg/dL and 17 patients had plasma cortisol levels >5 μg/dL. Plasma cortisol level was not suppressed in 4 of 9 patients in Group A and in 13 of 14 patients in Group B (p=0.013). Nighttime salivary cortisol level was measured in all patients. The mean nighttime salivary cortisol level was 0.189 ± 0.99 μg/dL in the non-SCS group, 0.268 ± 0.137 μg/dL in Group A, and 0.266 ± 0.157 μg/dL in Group B, respectively. It was significantly higher in Groups A and B than in the non-SCS group (p=0.001). There was no significant difference between Groups A and B in terms of mean nighttime salivary cortisol level (p=0.990).Comparison of statistically significant results between non-SCS group, Group A, and Group B is shown in Table 2. When the cut-off value of plasma cortisol levels after the 1-mg DST was considered ≥1.8μg/dL, the sen-

Table 1. Demographic characteristics of non-SCS and SCS groups

Parameters (n)	Non-SCS	SCS	P-value
Age (129)	55.2±10.1	61.4±10.2	0.001
BMI (95)	32.3±6.2	30.5±6.0	0.193
ACTH (128)	19.6±11.4	19.6±11.5	0.907
Basal cortisol (129)	13.0±4.5	15.7±4.1	0.001
DHEA-S (116)	82.5±63.1	75.6±75.7	0.161
Nighttime salivary cortisol (129)	0.18±0.09	0.26±0.14	0.001
Nighttime serum cortisol (11)	-	7.5±4.3	-
2-mg DST (23)	-	3.29±1.9	-
LDL (103)	123.2±29.5	131.9±39.1	0.390
TG (104)	152.5±89.9	152.8±64.4	0.569
Mass size (129)	19.9±9.7	24.9±10.7	0.003

Table 2.Comparison of statistically significant results between non-SCS group, Group A, and Group B

Parameters	Non-SCS	Group A	Group B	P-value
Age	55.2±10.1	60.5±12.2	62.4±7.3	0.004
Basal cortisol (μg/dL)	13.0±4.5	16.7±4.0	14.4±4.0	<0.001
Cortisol after 2-mg DST (μg/dL)	-	2.09±1.26	4.07±1.90	0.013
Nighttime salivary cortisol (μg/dL)	0.189±0.99	0.268±0.137	0.266±0.157	0.001
Mass size (mm)	19.9±9.7	22.5±8.2	27.8±12.7	0.001
Prevalence of HT (%)	54.0	73.9	84.2	0.015

sitivity, specificity, and cut-off value of salivary cortisol levels were calculated as 68%, 57%, and 0.195 µg/dL by ROC curve analysis.The reference range for salivary cortisol level at our center was determined as 0.04-0.56 µg/dL (Figure 1). A second confirmatory test (baseline ACTH level <10 pg/ml, DHEA-S level <35 µg/dL, plasma cortisol level after the 2-mg DST ≥1.8 µg/dL, nighttime salivary cortisol level ≥0.56 µg/dL) was added to reevaluate the diagnosis of SCS. If the cut-off values of plasma cortisol levels after the 1-mg DST were considered ≥1.8 µg/dL, ≥3 µg/dL, and ≥5µg/dL, the detection rates of SCS were respectively 22.4% (n=29), 12.4% (n=16), and 3.8% (n=5).The same criteria were re-examined only by changing the cut-off value of salivary cortisol level.The cut-off value of salivary cortisol levels was considered 0.195 µg/dL in our study. According to this, if the cut-off values of plasma cortisol levels after the 1-mg DST were considered ≥1.8 µg/dL, ≥3 µg/dL, and ≥5µg/dL, the detection rates of SCS were respectively 28.6% (n=37), 14.7% (n=19), and 4.6% (n=6).

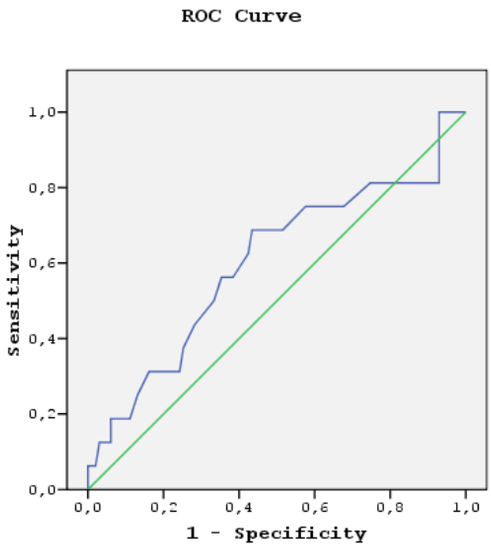

Figure 1. Sensitivity and specificity curves determined for salivary cortisol in the patients having plasma cortisol levels ≥1.8µg/dL after the 1-mg DST

Discussion

The vast majority of adrenal incidentalomas are non-functional benign cortical adenomas. Recent studies have shown that some benign tumors may also be functional at a subclinical level. Depending on the diagnostic criteria used, the prevalence of SCS ranges from 5% to 20% [7]. In our study, we found that it varied between 4.6% and 28.6% according to different cut-off values of plasma cortisol levels.

In previous studies, patients with adrenal incidentalomas were in the middle- and older-age groups. The mean age of the patients participating in our study was 57.2 years. The mean age was found to be statistically significantly higher in the patients with SCS than in the patients without SCS. Accordingly, we think that incidental adrenal masses in older patients may be more functional in terms of cortisol hypersecretion.When the metabolic and biochemical parameters were assessed, we could not detect any significant difference between the groups in terms of parameters such as obesity, diabetes, cholesterol, triglyceride, DHEA-S, plasma ACTH, osteopenia, and osteoporosis. When the literature was examined, conflicting results were found for these parameters. The prevalence of hypertension was %84 in the patients having plasma cortisol levels ≥3µg/dL after the 1-mg DST (Group B) and 54%in the patients having plasma cortisol levels <1.8µg/dL after the 1-mg DST (the non-SCS) (p=0.015). Studies have also found that hypertension was more frequent in patients with SCS [8]. In accordance with the literature, basal cortisol level was also found to be higher in the patients with SCS in our study (p=0.001).

In our study, we found that the mean tumor size was significantly greater in the patients having plasma cortisol levels ≥3 µg/dL after the 1-mg DST (Group B) than in the patients having plasma cortisol levels <1.8µg/dL after the 1-mg DST (the non-SCS) (p=0.001). Many studies have shown a significant association between tumor size and cortisol hypersecretion [9]. This suggests that intrinsic secretory activity in tumor cells, the amount of cortisol produced, and gaining autonomy are associated with tumor size.

In the literature, the prevalence of SCS has been reported to be between 5% and 20% depending on different diagnostic criteria [10]. In our study, we determined that the prevalence of SCS varied between 3.8% and 22.4% based on different diagnostic criteria. We also determined that it varied between 4.6% and 28.6% based on the same diagnostic criteria when the cut-off value of salivary cortisol levels was considered 0.195 µg/dL. In our study, we found that the patients having plasma cortisol levels ≥3 µg/dL after the 1-mg DST (Group B) were older and had a greater mass and a higher prevalence of hypertension. We think that plasma cortisol cut-off values greater than 1.8 µg/dL after the 1-mg DST should be used in patients with adrenal incidentalomas. Studies have shown that the 2-mg 2-day DST is more specific for cortisol hypersecretion than the 1 mg DST. However, the first test has been not preferred in many studies due to difficulty in its administration [11]. In our study, 23 patients underwent the 2-mg DST. After the 2-mg DST, 6 patients had plasma cortisol levels <5 µg/dL and 17 patients had plasma cortisol levels >5 µg/dL. Plasma cortisol level was not suppressed in 4 of 9 patients in Group A and in 13 of 14 in Group B (p=0.013). We revealed that the 2-mg 2-day DST is a more specific test for the diagnosis of SCS. Due to the limitations in midnight serum cortisol measurement, many researchers have suggested additional hormone tests for the diagnosis of SCS. One study found that the sensitivity, specificity, and cut-off value of midnight salivary cortisol levels were 22.7%, 87.7%, and 0.18µg/dL. Another study found that the sensitivity, specificity, and cut-off value of midnight salivary cortisol levels were 66%, 69.1%, and 0.17 µg/dL [12-13]. Similarly, we found that the sensitivity, specificity, and cut-off value of salivary cortisol levels were calculated as 68%, 57%, and 0.195 µg/dL. The normal reference range of the kit used in our study was 0.04-0.56 µg/dL. For this reason, we have demonstrated that it is necessary to determine cut-off values specific to different populations.

Conclusions

Greater tumor size, older age, and deteriorated metabolic parameters increase the risk of development of SCS in patients with adrenal incidentalomas. As the cut-off value of plasma

cortisol level used in the 1-mg DST decreased, the incidence of SCS increased significantly. This leads to overdiagnosis of SCS in asymptomatic patients with adrenal incidentalomas. We think that plasma cortisol cut-off values greater than 1.8 µg/dL after the 1-mg DST should be used in patients with adrenal incidentalomas. New cut-off values should be determined for salivary cortisol level according to different populations.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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